

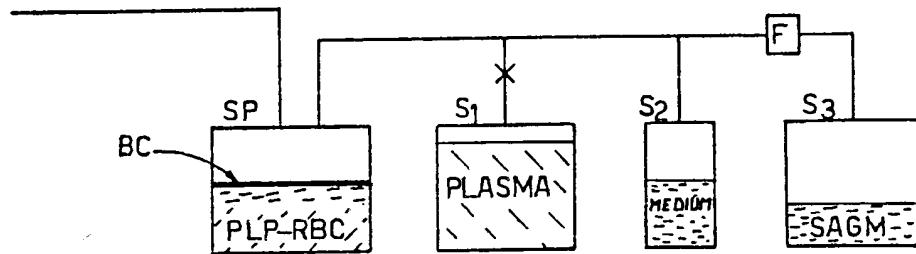


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(54) Title: PROCEDURE AND CONTAINER FOR THE PREPARATION AND STORAGE OF PLATELET CONCENTRATES



## (57) Abstract

A procedure for the routine preparation in closed system and storage in a medium made of natural colloid (plasma) and crystalloid solution of PC deprived of leukocytes, which is based on a quadruple blood bag (PB) containing CPD anticoagulant, an empty satellite bag (S1), a satellite bag (S2) containing a synthetic crystalloid solution, a satellite bag with SAGM (or similar) additive solution for red blood cells (RBC), consisting in collecting the blood in the primary bag (PB), in centrifuging the blood bag at high speed, in expressing the supernatant plasma into bag (S1), in collecting 15-20 mL of the buffy-coat (BC) into bag (S2), in adding the SAGM solution of bag (S3) into bag (PB) containing the RBC concentrate, in disconnecting bags (PB) and (S1) from bags (S2) and (S3), in centrifuging bag (S2) at soft spin and in transferring the supernatant platelet concentrate (PLP-PC) formed after this centrifugation into the empty bag (S3), through a cotton wool filter (F) inserted along the tube line so as to remove residual leukocytes which contaminate the PC.

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Procedure and container for the preparation and storage of platelet concentrates.

This invention relates to a procedure for the routine preparation in closed system of platelet concentrates (PC) deprived of leukocytes and stored in a medium consisting of a mixture of natural colloid (plasma) and of crystalloid solution (called 5 thereafter "medium"), and in particular for PC storage.

Standard RBC concentrates and PC, prepared by serial centrifugation from multiple bags and storage in a natural colloid medium (plasma), are contaminated with plasma and leukocytes, which can determine acute and chronic side-effects in transfusion 10 recipients.

The transfusion of platelet concentrates (PC) is necessary for preventing the risk of hemorrhage in patients with low platelet count (thrombocytopenia).

Routine production of blood components (RBC and PC) with higher 15 purity is hampered by technical difficulties concerning particularly the separation and storage of blood cells.

Presently available methods for preparing PC are based on soft spin of whole blood donations, the collection of platelet-rich-plasma, and the concentration of platelets in 50 mL plasma by high 20 speed centrifugation. Such PC can be stored for a maximum of 5 days. A different approach is the collection of buffy-coats (BC) of whole blood donations (the BC is the layer formed at the red blood cell-plasma interface after whole blood high-speed centrifugation), followed by soft spin of the BC and collection of 25 platelets in the supernatant. The principal untoward effect of PC transfusion is the production of alloantibodies by PC recipients, caused by leukocytes which contaminate the PC. Moreover, allergic reactions to plasma proteins can occur.

To prevent these complications and to improve the quality of 30 platelet transfusion the invention described below was developed.

The aim of this invention is to remove this difficulty by means of a procedure for blood fractionation allowing the routine

production and storage in closed system of plasma- and leukocyte-poor RBC concentrates (PLP-RBC, ie RBC containing less than 10% of donated plasma and less than 30% of donated leukocytes), and of plasma- and leukocyte-poor platelet concentrates (PLP-PC, ie PC in a medium containing about 15-40% plasma, and free of leukocytes detectable by microscope counting).

In the procedure reported in this invention an additive RBC solution is used (SAGM or similar), in addition to a synthetic crystalloid solution which is added to the platelets so as to 10 improve their quality and prolong storage to about 10 days.

The above crystalloid solution is preferably made (considering, for example, a volume of 40 mL) of 5.26 g sodium chloride; 2.22 g sodium acetate; 5.02 g sodium gluconate; 0.37 g potassium chloride; 0.14 g magnesium chloride; H<sub>2</sub>O for IV use qs to 1,000 15 mL, pH 7.4 ± 0.1. It must be emphasized that this solution is very similar to one of the crystalloid solutions reported in the patents issued under the names of Gail Ann Rock and George Adams (Canada, Plasma-free medium for platelet storage, application, Nov 1, 1982, 414,583; USA, Plasma-free medium for platelet storage, 20 Pat 4,447,415; Europe, Plasma-free medium for platelet storage, Granted May 16, 1984 - 0108588, Oct 31, 1983, 83306631.9; Canada, Improved plasma-free medium for platelet storage, application, July 20, 1984, 459,369); however, in the invention described here 25 the quality of platelets is improved since the crystalloid solution is fortified with an amount of plasma derived from the BC; this amount is between 10 and 40% of the final medium, preferably 20-30%.

The procedure is carried out with a multiple bag, preferably a quadruple bag consisting of a primary bag and 3 satellite bags. 30 After high speed centrifugation, the blood contained in the primary bag is separated into a red blood cell button and supernatant plasma; this latter is transferred into one of the

satellite bags, while the SAGM solution is added to the primary bag for red blood cell storage, after removing an amount of the buffy-coat (BC) layer, which is the layer between red blood cells and plasma, a layer enriched of platelets. The buffy-coat is 5 transferred into a second satellite bag containing the crystalloid solution and, after a soft spin, the supernatant platelet concentrate is transferred in the third, empty satellite bag, through a cotton wool filter inserted along the tubing, which is capable of removing residual leukocytes which contaminate the 10 platelet concentrate. A platelet concentrate prepared according to this method can be stored for about 10 days in this bag, which is made of plastic material with gas exchange characteristics similar to those of polyolefin.

The above procedure and bag system for the preparation of platelet 15 concentrates from buffy-coats (BC) and their storage in a crystalloid colloid medium can be used also with BC prepared with standard blood bag systems, by mixing 6-8 BC in a transfer bag containing an appropriate amount of the above crystalloid solution. After a soft spin, the supernatant, containing the 20 platelets, is transferred through a cotton wool filter into a new transfer bag where it is stored.

The procedure derived from this invention is now described, just for the sake of example, and with no limitation to its implementation, with the aid of enclosed fig. 2 through 5, which 25 describe the procedure steps, as performed with a quadruple blood bag, for the preparation and storage of PLP-PC and PLP-RBC from a blood donation.

The multiple blood bag consists of a primary bag PB and of 3 satellite bags S1, S2, S3, integrally connected to each other with 30 flexible plastic tubes.

The primary bag PB contains CPD (citrate-phosphate-dextrose) anticoagulant, the satellite bag S1 is empty, the satellite bag

S2, which is cylinder-shaped and has a volume of 40-60 mL, contains 40 mL of crystalloid solution, the satellite bag S3 contains the SAGM red blood cell additive solution, and is made of plastic material with gas permeability characteristics similar to 5 polyolefin. Along the tube connecting bags S2 and S3 a small (2-3 g) cotton wool filter is inserted.

The procedure regarding this invention is carried out as described below.

The blood donation is collected in the primary bag PB through a 10 collection line I, at one end of which the blood collection needle is attached, and mixed with the CPD anticoagulant (fig. 1). The blood collected in the primary bag PB is centrifuged at high speed, e.g. 3,800 rpm for 4 min in a Sorvall RC-3B centrifuge. After this centrifugation, plasma- and leukocyte-poor red blood 15 cells are concentrated in the lower part of the primary bag PB, the supernatant plasma is contained in the higher part of the bag, and in the layer between plasma and red blood cells the vast majority of platelets are concentrated. This small layer, enriched of platelets, which separated red blood cells from supernatant 20 plasma is defined "buffy-coat" (BC)(fig. 2).

The supernatant plasma (except for about 30 mL) is expressed into and stored in satellite bag S2, which is disconnected from the other bags (fig.2).

A total of 25-50 mL of BC are then collected, which contain about 25 5 g hemoglobin, 80% of donated platelets, 50% of donated leukocytes and 20-40 mL plasma, and expressed into satellite bag S2, which contain the crystalloid medium (fig. 3).

Preferably, the crystalloid solution contained in satellite bag S3 has the following composition (reference to a volume of 40 mL): 30 5.26 g sodium chloride; 2.22 g sodium acetate; 5.02 g sodium gluconate; 0.37 g potassium chloride; 0.14 g magnesium chloride; H<sub>2</sub>O for IV use qs to 1,000 mL, pH 7.4 ± 0.1.

The SAGM solution contained in satellite bag S3 is added to the red blood cell concentrate PLP-RBC in the primary bag PB for its storage. The primary bag, containing the PLP-RBC and the satellite bag S1 containing the plasma are disconnected from satellite bags 5 S2 and S3, which are stored, integrally connected to each other, in a platelet tumbler (a rotating machine for platelet resuspension during storage) (fig.4).

The buffy-coat (containing the platelets) contained in satellite bag S2 can be stored at room temperature for a maximum of 48 h. If 10 platelet concentrates are needed the buffy-coat is centrifuged at low speed, so as to concentrate the red blood cells and the leukocytes in the lower part of the bag S2, and to collect the leukocyte-poor platelet concentrate in the higher part of satellite bag S2. The cylinder shape of satellite bag S2 favors a 15 good separation between the 2 layers above after the soft spin.

The supernatant PLP-PC is transferred from satellite bag S2 to satellite bag S3 (which had been previously emptied of the SAGM solution), through filter F, which removes residual leukocytes present in the platelet concentrate (fig. 5).

20 Filter F can be much smaller than traditional cotton wool filters for leukocyte removal (2-3 g instead of 17 g) because leukocyte contamination of platelet concentrates prepared from buffy-coats is much lower than that of platelet concentrates prepared with traditional methods, in which centrifugation is carried out on 25 platelet-rich-plasma instead of platelet-rich-buffy-coat.

Experimental data indicate that the platelet concentrate PLP-PC prepared with the above method can be stored in the crystalloid/colloid medium for at least 10 days in a bag made of polyolefin, or of a plastic material capable of providing a gas 30 permeability similar to that of polyolefin, with a final pH of about 7.0, hypotonic shock response of 50% and morphology score according to Kunicki of 220. Moreover, PC prepared with this

method and invention have the following parameters after 7 days of storage: glucose consumption  $1.5 \text{ mmol/L}/10^9$  platelets (compared to 2.8 of PC prepared in the medium of G. Rock), and lactate production of  $3.56 \text{ mmol/L}/10^9$  platelets (compared to 6.09 of PC prepared in the medium of Rock). Both parameters suggest better platelet quality in our medium. In addition, platelet aggregation is better in our medium compared to that in the medium of Rock. These excellent results are mainly due to the crystalloid/colloid medium, of which a preferential composition has been given above, 10 in which a crystalloid solution is mixed with a small amount of plasma (natural colloid) present in the buffy-coat, from which the platelet concentrate is prepared.

The above procedure for the preparation of platelet concentrates from buffy-coats (BC) and their storage in crystalloid/colloid 15 medium can be also used with buffy-coats prepared from traditional blood collection bags, e.g. by using a triple bag made of a primary bag and 2 satellite bags, in which the red blood cell concentrate, the plasma and the buffy-coat are collected, respectively.

20 In this case 6-8 buffy-coats prepared with the methods above or similar are collected in a transfer bag of about 600 mL volume, containing about 350 mL of the previously described crystalloid solution. After soft spin, the platelet-rich supernatant is transferred through a small (2-3 g) cotton wool filter F into a 25 transfer bag of about 1000 mL volume, with the same characteristics of previously described bag S3, where it is then stored.

The procedure described in this invention offers the following advantages, in addition to those already reported, in comparison 30 to traditional procedures:

- leukocyte contamination of platelet concentrates is below detectable limits in all platelet concentrates, which implies a

series of outstanding advantages for the patients;

- platelet metabolism can be conditioned by modifying the storage medium with the addition of possible additives;
- there is no need to follow ABO compatibility between donor plasma and recipient red cells, due to the small amount of plasma present in the platelet concentrates;
- more plasma can be recovered from standard blood donations;
- it is possible to prepare platelet concentrates only upon request, so reducing the workload;

10 - the procedure favors the routine production of buffy-coat deprived red blood cell concentrates (which are of better quality than standard red blood cell concentrates), since it is based on the availability of buffy-coats.

Moreover, the crystalloid solution does not contain glucose, thus 15 reducing the potential risk of bacterial contamination of PLP-PC. The above advantages are supported by a series of in-vitro and in-vivo evaluations of platelet concentrates prepared from buffy-coats and stored in medium. A set of these evaluations is reported below.

20 We compared platelet concentrates prepared from pools of buffy-coats removed from standard blood donations and stored in the crystalloid solution described above (BCS-PC), with standard platelet concentrates, prepared from platelet-rich-plasma (PRP-PC). Total cell counts (median and range, n=43) in BCS-PC units 25 were: platelets  $3.6 \times 10^{11}$  (2.2-5.5), leukocytes  $21 \times 10^6$  (4-79); values of PRP-PC units (n=23) were: platelets  $0.64 \times 10^{11}$  (0.47-0.83); leukocytes  $148 \times 10^6$  (3-611). Since one BCS-PC unit was prepared from 7 blood donations, a meaningful comparison of PRP-PC and BCS-PC data can be made by multiplying PRP-PC data by 7. Both 30 platelet and leukocyte counts of BCS-PC were significantly lower than those of 7 PRP-PC. Platelet yield in BCS-PC and PRP-PC was 59 and 75 percent of donated platelets, respectively. Median number

of total leukocytes in 1 BCS-PC unit, prepared from a pool of 7 buffy-coats, was 50 times lower than that of 7 PRP-PC. In vitro parameters of adequate platelet quality were maintained for 10 days in BCS-PC stored in 1000 mL polyolefin bags. Prolonged 5 bleeding times were reduced or corrected in 3 of 3 thrombocytopenic leukemic patients evaluated before and after transfusion of stored BCS-PC. Pretransfusion, 1-h and 24-h posttransfusion median platelet counts of 57 leukemic recipients during 4 months of routine transfusion of BCS-PC (n=93) were 14, 10 35, and  $27 \times 10^9/L$ , compared with 13, 37 and  $31 \times 10^9/L$  of PRP-PC (n=246), respectively (p=ns). No reactions to BCS-PC were reported, compared with a reaction rate of 1.3 percent for PRP-PC transfusions. The median (and range) titer of ABO agglutinins in BCS-PC and PRP-PC was 8 (2-8) and 64 (32-128), respectively. A 15 detailed description of the results is reported in tables 1-3.

In vitro evaluation of BCS-PC and PRP-PC during storage is reported in table 1.

It can be seen (section A) that in vitro parameters of adequate platelet quality (pH above 6.0, morphology score above 200) were 20 maintained in BCS-PC for at least 10 days of storage in 1000 mL polyolefin bags; at this time the platelet response to hypotonic shock (HSR), a parameter related to platelet recovery and survival in normal volunteers, was still above 80 percent of values at day 1. This was not so for BCS-PC stored in 1000 mL PVC bags (section 25 B), in which the median pH value was below 6.0 at day 3. This was associated with a complete loss of HSR and a marked deterioration of platelet morphology. Similarly to other reports, PRP-PC stored in PVC satellite bags of 400 mL volume showed adequate quality preservation for at least 5 days (section C). On day 1,  $PO_2$  values 30 of BCS-PC in 1000 mL bags were about 2 times higher than those of PRP-PC in 400 mL bags, as expected on the basis of both a larger bag and a smaller platelet concentration in the former. Platelet

quality during storage in PVC deteriorated more rapidly in BCS-PC than in PRP-PC. A contributory cause could have been the smaller ratio between bag surface area and PC volume in the former, determining a less efficient gas transport during storage. Oxygen 5 consumption for aerobic metabolism, which is more favorable than anaerobic for PC preservation during storage, was maintained longest in BCS-PC stored in polyolefin, as indicated by the slowest  $\text{PO}_2$  rise.

Results of in-vivo study are reported in Table 2 and 3.

10 Pilot phase. Pretransfusion, 1-h and 24-h posttransfusion platelet counts and percent recovery values obtained in "stable" leukemic recipients are reported in table 2. It can be seen that values of BCS-PC stored for 1-8 days were similar to those of PRP-PC stored for 1-3 days. Bleeding time (reference values 2-7 min) was reduced 15 after BCS-PC transfusion in 3 of 3 patients evaluated, dropping from more than 30 to 9 min (6-day old BCS-PC containing  $3.0 \times 10^{11}$  platelets), from 25 to 5 min (4-day old BCS-PC containing  $2.8 \times 10^{11}$  platelets), and from 22 to 6 min (8 day old BCS-PC containing  $3.1 \times 10^{11}$  platelets). On these 3 occasions pre- and 1-h 20 posttransfusion platelet counts were 17-43, 16-56 and  $16-39 \times 10^9/\text{L}$ , respectively.

25 Routine phase. A total of 448 and 129 platelet transfusions were issued as 1-3 day-old PRP-PC and 1 day-old BCS-PC, respectively, to 57 leukemic patients. This group included all hematological patients transfused with platelet concentrates in the study period. Pretransfusion, 1-h and 24-h posttransfusion platelet count and percent recovery, available on 55 percent (PRP-PC) and 72 percent (BCS-PC) of occasions, are reported in table 3. Increments of BCS-PC were not significantly different from those 30 of PRP-PC. No reactions were reported for BCS-PC, compared with 6 for PRP-PC; of these, 2 were febrile and 4 allergic.

Table 1 storage.

Day	pH	PO <sub>2</sub> (mm Hg)	PCO <sub>2</sub>	HSR (%)	Morphology score	Platelet count (% of day 1)
<b>A) BCS-PC units stored in 1000 mL polyolefin bags (n=8)</b>						
1	6.9 (6.7-6.9)	87 (70-109)	23 (20-31)	62 (39-68)	311 (250-333)	100
3	6.9 (6.4-6.9)	73 (61-115)	26 (19-36)	64 (46-67)	273 (230-306)	95 (75-100)
5	6.8 (6.4-6.9)	93 (40-123)	28 (19-39)	62 (42-70)	266 (215-297)	88 (73- 96)
10	6.7 (6.3-7.0)	101 (76-145)	25 (20-36)	51 (33-69)	203 (185-260)	81 (71- 93)
15	6.9 (5.8-7.2)	122 (65-175)	17 ( 9-27)	31 ( 0-60)	180 (105-280)	76 (68- 81)
<b>B) BCS-PC units stored in 1000 mL PVC bags (n=8)</b>						
1	6.8 (6.7-6.9)	90 (82-124)	27 (18-31)	50 (42-67)	312 (270-329)	100
3	5.9 (5.8-6.5)	148 (71-161)	37 (24-69)	0 (0-69)	160 (142-231)	92 (88- 97)
<b>C) PRP-PC units stored in 400 mL PVC bags (n=10)</b>						
1	7.0 (6.7-7.1)	40 (28-149)	56 (19-90)	55 (30-69)	260 (210-331)	100
3	7.0 (6.2-7.2)	47 (16-81)	46 (34-77)	51 (36-56)	226 (122-331)	100 (100-100)
5	7.0 (5.6-7.1)	58 (13-154)	42 (16-48)	47 (0-60)	208 (92-263)	100 (81-100)
10	6.6 (5.5-7.0)	86 (50-199)	35 (13-42)	21 (0-40)	167 (71-195)	94 (88- 97)
15	6.1 (5.7-6.8)	181 (123-201)	14 (6-28)	0 (0-9)	107 (73-154)	72 (60- 94)

Table 2. In vivo study: pilot phase. Median (and range) platelet counts and percent recovery in 11 "stable" leukemic patients receiving both BCS-PC and PRP-PC.

Type of PC	Storage	Days	Bag	Platelet count ( $\times 10^9/L$ )		Platelet in vivo recovery	
				Pre-	Posttransfusion	1-h	24-h
BCS-PC n=8	1000 mL PVC	13 (9-20)		42 (23-56)	34 (28-46)	59 (19-81)	44 (28-62)
BCS-PC n=8	1000 mL polyolefin	17 (9-21)		43 (16-56)	33 (12-40)	50 (0-102)	36 (0-54)
BCS-PC n=5	1000 mL ~ polyolefin	10 (5-21)		43 (13-91)	21 (12-28)	73 (17-161)	11 (7-40)
PRP-PC n=15	400 mL PVC	16 (4-29)		50 (10-92)	37 (14-79)	49 (0-102)	30 (0-92)

Table 3. In vivo study: routine phase. Median (and range) platelet counts and percent recovery in 57 hematologic patients receiving platelet transfusion during 4 months of routine use of both BCS-PC and PRP-PC.

Type of PC	Storage	Days	Bag	Platelet count ( $\times 10^9/L$ )		Platelet in vivo recovery	
				Pre-	Posttransfusion	1-h	24-h
BCS-PC n=93	1000 mL PVC	14 (2-40)		35 (7-83)	27 (3-64)	40 (0-103)	22 (0-98)
PRP-PC n=246	400 mL PVC	13 (1-69)		37 (4-114)	31 (1-137)	31 (0-110)	20 (0-124)

It is emphasized that the invention is not limited to the particular description reported above and in the enclosed figures, but it can be modified in several minor parts within the scope of the invention.

CLAIMS

1. A method for the preparation and storage of plasma- and leukocyte-poor platelet concentrates from buffy-coats, which comprises the transfer of at least 1 buffy-coat into a bag (S2) 5 containing a synthetic crystalloid solution (which is diluted with the plasma contained in the buffy-coat so as to constitute a colloid/crystalloid medium for platelet storage), the soft spin of the bag (S2) and the transfer of the supernatant PLP-PC formed after centrifugation into a (S3) bag made of polyolefin or of 10 plastic material with gas permeability similar to polyolefin, through a cotton wool filter (F) of 2-3 g, so as to remove residual leukocytes contained in the platelet concentrate.
2. A method as claimed in claim 1, wherein the above bag S2 has an elongated, cylinder shape.
- 15 3. A process according to claim 1, wherein 1000 ml. of said synthetic crystalloid solution contain 5.26 gr sodium chloride, 2.22 gr sodium acetate, 5.02 gr sodium gluconate, 0.37 gr potassium chloride, 0.14 gr magnesium chloride,  $H_2O$  for I.V. use qs to 1000 ml and wherein said crystalloid solution has a pH of 20 7.4  $\pm$  0.1.
4. A process according to claim 1, wherein 6-8 BC obtained from as many donations are transferred into the bag (S2).
5. A process according to claim 1, wherein the PLP-PC are stored in a synthetic crystalloid / natural colloid solution, comprising 25 the crystalloid medium of bag (S2) and the plasma of each BC (about 20-40 ml of plasma in a BC volume of 25-50 ml).
6. A process according to claim 1, wherein the BC are separated in a multiple bag for the preparation and storage of PLP-PC and PLP-RBC (plasma- and leukocyte- poor RBC concentrates), e.g. in a 30 quadruple blood bag comprising a primary bag (PB) and three satellite bags (S1), (S2), (S3).
7. A process according to claim 6, comprising:

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collecting the blood in a primary bag (PB) containing CPD anticoagulant, centrifuging at high speed said primary (PB) bag, transferring the supernatant plasma into the bag (S2) containing the crystalloid solution, and transferring the SAGM solution of bag 5 S3 into the (PB) bag containing PLP-RBC.

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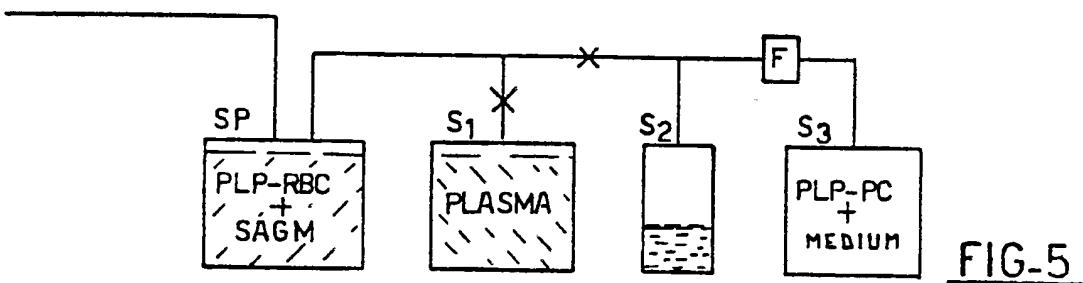
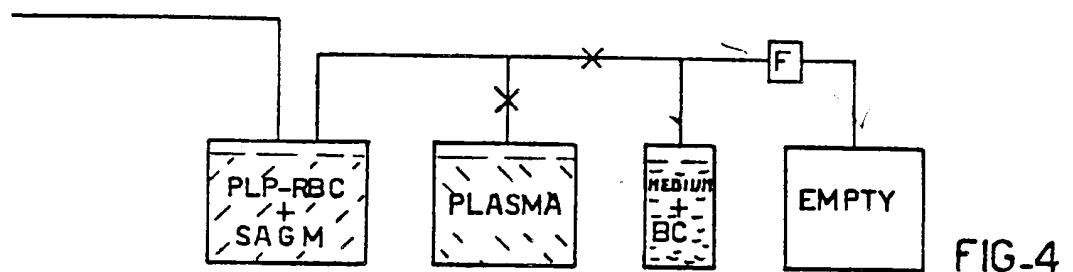
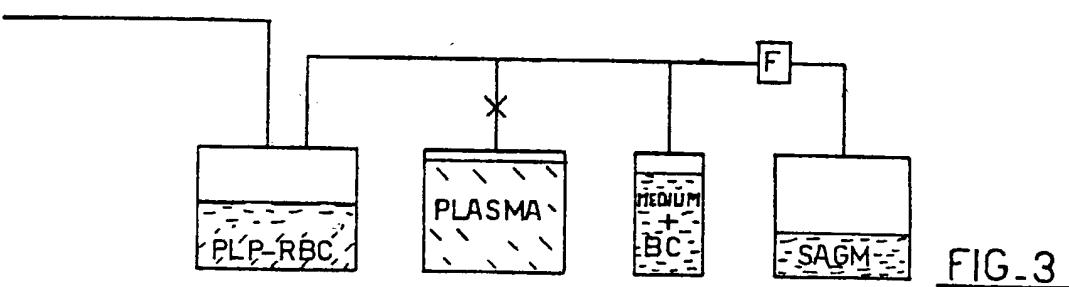
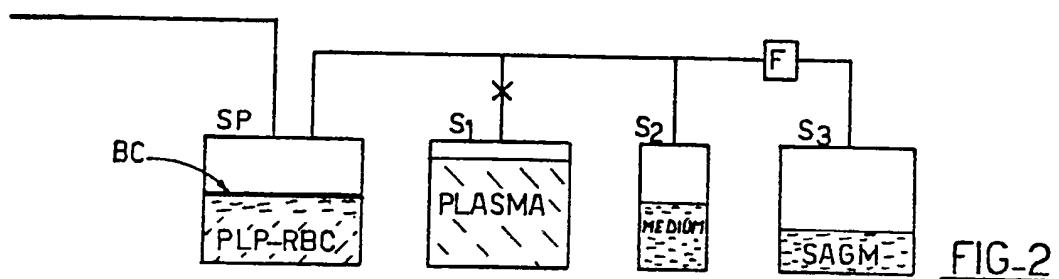
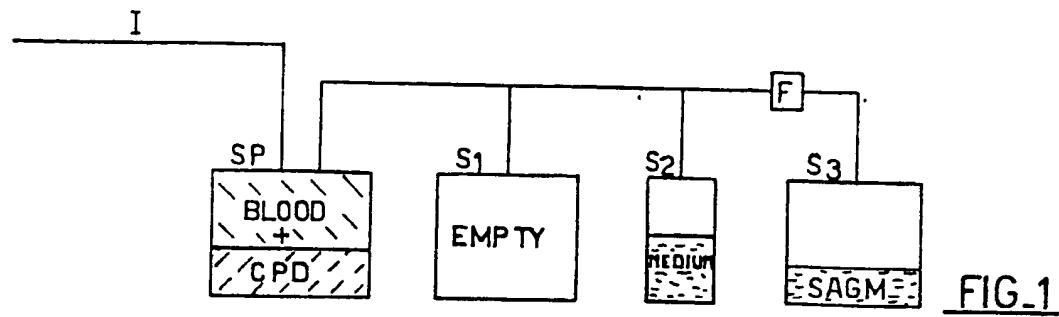
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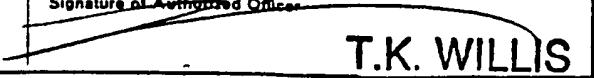
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# INTERNATIONAL SEARCH REPORT

International Application No PCT/IT 89/00048

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>8</sup> According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : A 61 K 35/14, A 61 M 1/36		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	A 61 K, A 61 M	
<b>Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched</b> <sup>9</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>10</sup>		
Category <sup>11</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	EP, A, 0233112 (TERUMO K.K.) 19 August 1987 see page 4, lines 32-36; page 11, lines 31-36; page 12, lines 22-29; page 20, lines 28-34 --	1
A	US, A, 4639373 (B.M. BABIOR) 27 January 1987 see column 2, lines 18-19 --	1
A	US, A, 4447415 (G.A. ROCK) 8 May 1984 see claims 1,8 cited in the application --	1
A	WO, A, 86/03122 (CURATECH INC.) 5 June 1986 see claim 1 --	1
A	US, A, 4344936 (G. SOSLAU) 17 August 1982 see claim 1 -----	1
<p><b>* Special categories of cited documents:</b> <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
13th September 1989	09.10.89	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE		

ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

IT 8900048

SA 29644

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 29/09/89. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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